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THE SITE OF ACTION OF INERT DUSTS ON CERTAIN BEETLES INFESTING STORED PRODUCTS

By V. B. Wigglesworth, M.D., F.R.S., F.R.E.S.

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It has been known for many years that beetles infesting grain and similar materials are killed by contact with certain inert dusts, notably finely ground siliceous materials, and that the cause of death is desiccation (Alexander, Kitchener and Briscoe (1944), Parkin (1944)). From experiments with the bug *Rhodnius prolixus* Stål, and with larvae of *Ephestia kuehniella* Zeller and *Tenebrio molitor* L., it was concluded that these materials act by the abrasion of the thin coating of wax which normally renders the cuticle impermeable to water (Wigglesworth, 1945). These observations have now been extended to some of the common beetles infesting stored products in order to demonstrate what areas of the body surface in such insects are subject to abrasion.

The method used was the same as that already described. The abraded areas are revealed by the fact that when the waxy covering of the cuticle is removed, an underlying layer is exposed that contains materials (probably dihydroxyphenols) which reduce ammoniacal silver hydroxide. Consequently if the treated insect is immersed in ammoniacal silver solution the affected spots stain a deep brown or black.

In small, darkly pigmented insects it is often difficult to see clearly the silver stained areas. It was therefore necessary to use a bleaching agent that would decolorise the cuticle without removing the silver deposit. For this purpose hydrogen peroxide (90/100 volumes) was used. The beetles were kept for forty-eight hours at 25° C. in glass capsules lightly dusted with alumina. By this time most of them were dead or moribund. They were immersed in 5 per cent. ammoniacal silver hydroxide for one hour, washed thoroughly with distilled water, fixed for one hour in Carnoy's fixative and then placed in the strong hydrogen peroxide solution until they were a pale amber or straw colour. This usually required two or three days. They were then washed, dehydrated in alcohol, cleared in beechwood creosote and mounted whole or after dissection in Canada balsam.

The following species were studied : *Tenebrio molitor* L., *Rhizopertha dominica* Fabricius, *Calandra granaria* L., *Anobium punctatum* de Geer, *Tribolium confusum* Jaquelin du Val and *Ptinus tectus* Boield. There was considerable variation in the extent of the silver staining areas in different species and in different individuals of the same species. The specific differences may be due in part to differences in activity. An active insect like *Tribolium* or *Calandra* commonly shows more extensive abrasion than a sluggish insect like *Anobium*.

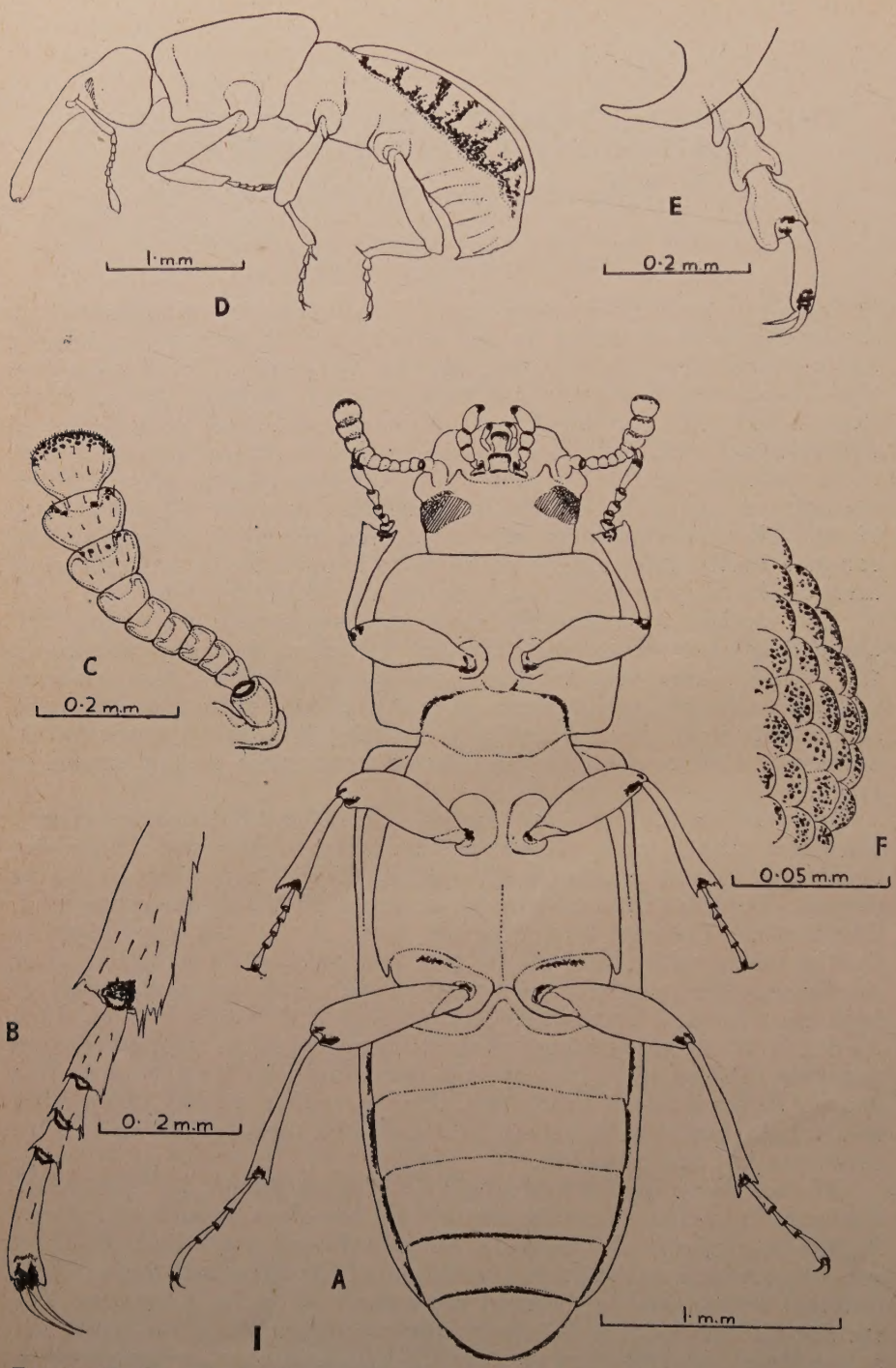

FIG. 1.—Beetles stained with ammoniacal silver hydroxide and bleached with hydrogen peroxide to show the distribution of the abraded areas. *A*, *Tribolium confusum*; *B*, posterior tarsus of the same; *C*, antenna of the same; *D*, *Calandra granaria* with the left elytron removed; *E*, detail of tarsus of the same; *F*, corneal facets of *Rhizopertha dominica*.

But the main results were consistent throughout. Fig. 1, *A*, shows a typical example in *Tribolium*. It can be seen that there is no abrasion of the strongly sclerotised areas of the elytra, of the ventral surface of the abdomen or of the head, legs, etc. But where the dust has come into contact with the soft cuticle of the dorsum of the abdomen along the margins of the elytra, there is well-marked silver staining. There is staining also in the intersegmental articulations of the legs, particularly of the tarsi (fig. 1, *B*). The same is true of the terminal segment and the articulations of the maxillary and labial palps. There is some staining of the distal segments of the antennae, particularly on their ventral aspects, and a very conspicuous stained ring where the flagellum articulates with the pedicel (fig. 1, *C*). The surface of the ligula is extensively abraded and so are the various sutures on the ventral aspect of the head. The corneal facets of the compound eye also are stained with the silver. The membrane between the pro- and mesothorax is abraded. The intersegmental membranes between the abdominal sternites are not affected, with the exception of the rather wide protruding folds of soft cuticle behind segments 4 and 5.

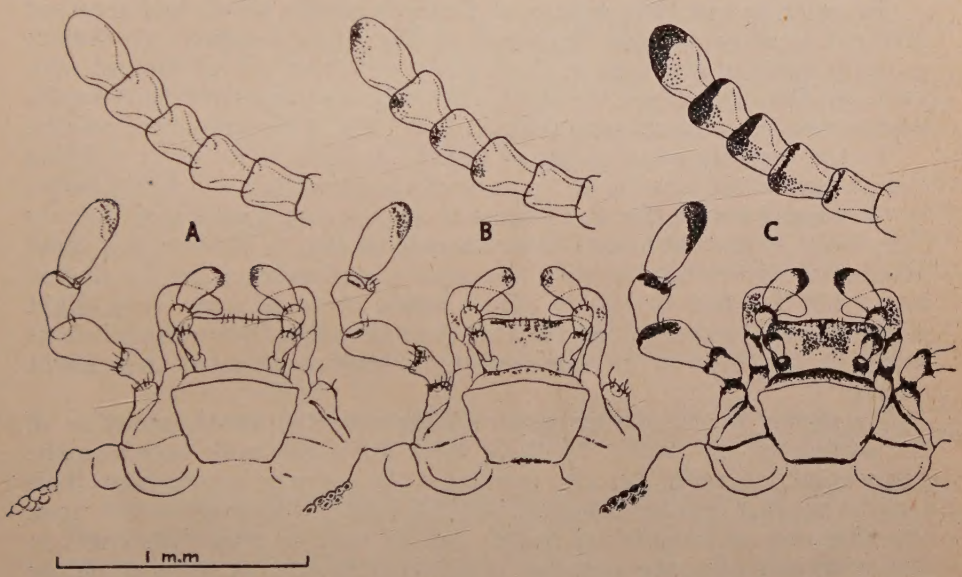

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FIG. 2.—Ventral view of mouth parts and apical segments of antenna of *Tenebrio molitor*, stained with ammoniacal silver hydroxide and bleached with hydrogen peroxide. *A*, recently moulted adult kept in clean glass container without food ; *B*, old adult from stock culture ; C, adult after two days in contact with alumina dust.

In brief, it is evident that abrasion has occurred at all those points where there is soft cuticle which is moved in contact with the dust. Such abrasion may result from the rubbing of the insect against the dust on its surroundings as it moves about (for example, the surface of the eye, of the ligula, the ventral sutures of the head, perhaps the margins of the dorsum of the abdomen and the membranes behind the

4th and 5th segments). Or the abrasions may result from the movements of the articulations. For this to occur, the joints must be so constructed that the fine dust can get into them, and they must move. Thus the more open character of the articulation between the flagellum and the pedicel, and the greater amount of movement that occurs there, probably account for the conspicuous abraded ring at this point in *Tribolium*.

In *Tenebrio* the distribution of abraded areas is very similar. In the other insects it was usually less extensive but had substantially the same distribution. In some individuals of *Calandra* the dust had got below the elytra, and the dorsum of the abdomen was extensively abraded (fig. 1, *D*).

In the untreated control insects there is usually no silver staining anywhere. But in *Tribolium* the extreme tips of the labial and maxillary palps stain, and there is sometimes a very small amount of staining at the base of the flagellum—suggesting that in the normal life of the insect abrasion may occasionally take place at certain points, as happens on a very extensive scale in insect larvae living in the soil (Wigglesworth, 1945).

In order to test this, groups of *Tenebrio* adults which had received different treatments were immersed in the silver solution. (i) Adults recently moulted and kept in clean glass containers out of contact with food. (ii) Old adults from the stock culture in bran and flour. (iii) Adults kept in contact with alumina for two days. Fig. 2 illustrates the results.

In the recently moulted insect (fig. 2, *A*) there is no silver staining except around the sensilla at the ends of the maxillary and labial palps. In the dusted insect (fig. 2, *C*) there is very severe abrasion as already described. In the old insects from the culture (fig. 2, *B*) there is a small amount of silver staining with the same distribution as in the dusted insect but far less in extent. Here again there is some very slight staining over the corneal facets. It is clear therefore that normal insects living in this type of environment may become abraded to a slight degree.

As shown in the earlier paper (Wigglesworth, 1945), abrasion of the surface of the cuticle by these inert dusts not only increases the permeability to water but also facilitates the entry of insecticides. This may be one factor in the influence which the filler is known to exert upon the efficiency of insecticidal dusts. And it may be that differences in the behaviour or in the anatomy of different insects, by influencing the amount and the distribution of abrasion, may account for some of the differences in susceptibility of insects to such dusts. If old insects commonly show some degree of abrasion in their normal environment, this might render them increasingly susceptible to insecticides.

Summary.

The abrasion of the protective wax layer in beetles kept in contact with fine alumina takes place chiefly in the articulations of the limbs, but to some extent also at other points where the soft cuticle of the moving insect is rubbed against the dust or where the dust gets into other moving joints. Normal insects living in flour or bran may show very slight amounts of abrasion with increasing age.

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Parkin, E. A., 1944, Control of the granary weevil with finely ground mineral dusts. *Ann. appl. Biol.* **31** : 84–88.

Wigglesworth, V. B., 1945, Transpiration through the cuticle of insects. *J. exp. Biol.* **21** : 97–114.

Book Notices.

The Tabanidae or Horseflies and Deerflies of Georgia. By P. W. Fattig. *Emory Univ. Mus. Bull.* **4** : 1–26, 1946.

This work forms part of a series covering the insects of Georgia, the Mutillidae, Phyllophaga, and Asilidae having been dealt with in the three previous Bulletins.

One hundred and eighteen species of Tabanidae are recorded in the present work, which also includes a note of the life history, economic importance and parasites and predators of Tabanids. The author recalls some field experiences with these insects, and concludes with a useful bibliography.

Insects of the Pacific World. By C. H. Curran. New York (Macmillan & Co.), 1946. Pp. xv+317, 7 pls., text illust. Price $3.75.

This book has been written as a popular guide to the insects of islands in the Pacific. It is the first work of its kind to attempt to cover most of the insects of the area in one volume, and is part of the *Pacific World Series* describing the natural history of the islands in that region.

There are fourteen chapters covering all the orders of insects, but those which are of economic importance or have a wide popular appeal, such as the Lepidoptera, are treated in greater detail. There is a chapter on insects and disease followed by instructions on collecting, preserving and shipping insects.

Butterfly Lives. By S. Beaufoy. 4to. London (Collins), 1947. Pp. 112, text illust. Price 12s. 6d.

The main feature of this work is the profusion of photographs (over 190 in number) from life, illustrating the life histories of twenty-two British butterflies.

The text describes the life history and habits of these species and throughout the author has included references to his own experiences in the collection and study of these insects, and in his introduction he expresses the hope that the work will serve to stimulate interest in the earlier stages of butterfly life, as well as in the perfect insects. The work is written in a style calculated to appeal to the beginner as well as to the more advanced entomologist.

A CATERPILLAR OCCURRING IN *NEPENTHES* PITCHERS (LEPIDOPTERA : NOCTUIDAE)

By Dr. A. Diakonoff.

(*Zoological Museum, Buitenzorg, Java.*)

Whilst the author was a prisoner of war at Pangkalan Balai, about thirty-five miles north from Palembang, on the S.E. coast of Sumatra, his fellow-prisoner, Mr. R. P. Lammers, of Buitenzorg, Java, brought him on one occasion some peculiar caterpillars. They had been found outside the camp in jungle which was being cleared by the prisoners of war to make place for a Japanese air-field. It was not the appearance of the caterpillars that attracted attention, but the fact that they chose as habitat the pitchers of a species of *Nepenthes*, which could not be identified at that time, but which probably was *N. gracilis* Korth. The pitchers were narrow, slightly compressed beneath, about 3 inches long and $\frac{1}{4}$ inch in diameter.

At first the author could not believe that there could exist any other relation between the pitchers and the insects than that of a food-plant and its parasites ; the caterpillars, which at first were taken for geometrid larvae, were quite ordinary-looking creatures ; neither did they differ from any Geometrid or Geometrid-like Noctuid larvae nor show any special adaptations to their strange abode. More peculiar, however, were the observations following this capture.

The larvae were light green, mixed with pale lilac in some places and about $\frac{1}{4}$ inch long.[1] They walked in Geometrid fashion, but they moved only when disturbed ; otherwise they remained motionless for long periods of time. Quite unexpected and striking was the fact that the larvae did not damage the *Nepenthes* plant at all, neither did they leave the pitchers. They remained motionless for hours on end, with one half of the body immersed in the fluid of the pitcher. It seemed to make no difference to them whether the head and fore part of the body or the hind extremities were thus submerged. They even preferred the position with the head and fore parts of the body pressed against the heap of animal remains at the bottom of the pitcher.

Without leaving the pitcher or damaging their tissues the larvae grew and one of them finally pupated. Mr. Lammers found a pitcher which contained another pupa. Both were dark brown and attached to the wall of the pitcher just above the surface of the fluid by a small whitish filament wound around the middle of the body. The moths emerged in about ten days (October 27th, 1944). One of them was brought along to London by the author and kindly identified by Mr. W. H. T. Tams, of the British Museum (Natural History), as *Eublemma radda* Swinhoe (1) of the subfamily Erastriinae, fam. Noctuidae.

[1] All the author's notes concerning measurements, colouring and rearing data of this insect were discovered by Japanese soldiers and destroyed.

This species, of which some material from Sarawak is preserved in the British Museum, was also obtained by C. Dover on Singapore Island (2) and identified by Mr. Tams (*ibid.*, p. 23). Mr. Dover writes, (p. 6): "A small Noctuid larva lives on *N. rafflesiana* and the larvae live and pupate on the smooth conducting surface within the pitcher, the moth nearly always escaping unharmed, as I have often found the empty pupal cases within the pitchers, but never a specimen of the adult moth." It is not clear from this paper whether Mr. Dover stated that the larvae fed on *Nepenthes* leaves; he surmised, however, that this *Eublemma* species would be connected with the genus *Nepenthes* in the same way as the Erastriine genus *Exyra* is connected with SARRACENIACEAE in S. America, on different species of which *Exyra* feeds.

The author is inclined to go much farther. He could not ascertain that the larvae of *Eublemma radda* caused any damage whatever to the *Nepenthes*. If they were common plant feeders, the damage they would do to the pitcher or to other parts of the leaf on account of the size of the larvae would be considerable and could not escape notice. But several larvae were reared for many days, they grew in the pitchers, and one of them pupated without feeding on the plant. The only plausible explanation is that the larvae are carnivorous scavengers and feed on insects' corpses, which are heaped in abundance on the bottom of every pitcher. The author did not observe the larvae feeding: probably they fed at night. In his opinion *Eublemma radda* is not only connected with the insectivorous *Nepenthes*, but shows a further stage of "parasitism" and adaptation to its habitat than the S. American *Exyra*: it leaves its host plant untouched, but feeds on its spoils.

The fact that other ERASTRIINAE are carnivorous and feed on coccids supports this surmise.

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A MUREXIDE TEST FOR THE RECOGNITION OF PTERINS IN INTACT INSECTS

By E. B. Ford, F.R.S.

(Reader in Genetics, University of Oxford.)

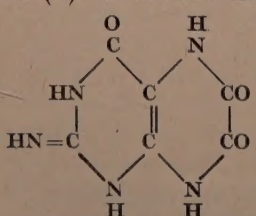
The ordinary murexide test for uric acid and its derivatives when applied to the wing-pigments of insects necessitates the destruction of the specimens. I find, however, that a murexide reaction can be obtained on an intact butterfly or moth. This makes it possible to distinguish the localisation of pterins in different components of the pattern. Moreover, the method is more sensitive than the normal one, and allows the recognition of these pigments even in a minute area of a single wing. It is therefore particularly useful in studying rare species.

I first noticed the reaction while making certain tests upon the butterfly *Delias eucharis* Drury. This is a large oriental species having a white ground colour. It belongs to the Pieridae, a family which includes the ordinary "white butterflies" (*e.g.*, the "Cabbage Whites") and the "Yellows" (*e.g.*, the Brimstone). On exposing a specimen to chlorine for twenty minutes, the white pigment was at first quite unaffected and remained so for several days, after which it changed in a few hours to a brilliant purple, producing a most striking effect. This colour has remained unaltered for two years when the insects are kept in the dark, but it fades considerably when exposed to strong light for a few days. It also fades to a certain extent after some months in several of the other species studied, even when light is excluded from them.

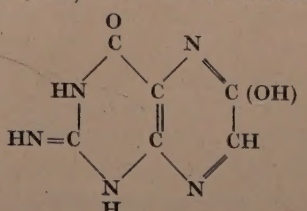
The delay in this reaction can be greatly reduced by fuming the specimen with ammonia after treatment with chlorine. A trace of the purple shade then appears almost immediately, and it is fully developed in a few hours. However, it is of a slightly more brownish tint when produced in this way.

Hopkins (3) had shown in 1895 that the white and yellow pigments of the Pieridae are compounds related to uric acid, and that these substances are restricted to this one family of butterflies so far as his tests extended : a generalisation which has so far remained unshaken. The white and yellow pigments of other Lepidoptera are entirely distinct from those of the Pieridae, and belong to a number of types which differ widely from one another.

Leucopterin, the white uric acid pigment of the pierine butterflies, was further examined by Wieland and others (10), and the less oxygenated yellow one, xanthopterin, was investigated by Schöpf and Becker (8). It is now known (5, 6, 7, 9, 11) that their structures are :

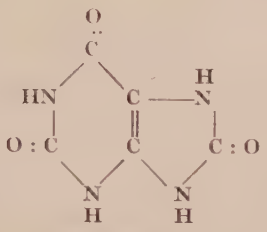


leucopterin
($C_5H_5O_3N_5$)



xanthopterin
($C_5H_5O_2N_5$)

The formula of uric acid is given also to indicate its close structural similarity :—



uric acid
($C_5H_4O_3N_4$)

Bearing these facts in mind, it seemed probable that the change from white to purple on the wings of *Delias eucharis*, following treatment with chlorine, is a form of the murexide reaction. I accordingly tested a number of additional species : 17 PIERIDAE, and 20 belonging to other families of butterflies. The purple colour was obtained with every one of the PIERIDAE and with none of the others. These results merit a brief examination.

The PIERIDAE which were studied in this work are listed in Table 1. They include representatives of each of the four main subfamilies, and within them those selected are well scattered from a classificatory point of view. The species tested which do not belong to the PIERIDAE

Table 1.—The species of the family PIERIDAE examined for pterins by means of the chlorine test. All gave a positive reaction.

Subfamily	Species
PIERINAE	*Aporia crataegi* L., *Delias eucharis* Drury, *Pieris brassicae* L., *P. rapae* L., *P. napi* L., *Anthocharis cardamines* L.
TERACOLINAE	*Colotis danae* Fb., *Hebomoia glaucippe* L.
COLIADINAE	*Colias croceus* Fourc., *Gonepteryx rhamni* L., *Terias brigitta* Cr.
DISMORPHIINAE	*Leptidea sinapis* L., *Pseudopieris nehemia* Bdv., *Enantia licinia* Cr., *E. melite* L., *Dismorphia thermesia* Godt., *D. nemesis* Latr.

(Table 2) are distributed among every one of the ten remaining families into which the butterflies of the world are usually divided. Consequently, as far as the numbers allow, the results demonstrate the proposition that the reaction here described is limited to those species

Table 2.—Species belonging to families other than the PIERIDAE which were examined for pterins by means of the chlorine test. None gave a positive reaction.

Family	Species
DANAIDAE	*Danaus chrysippus* L., *Amauris niavius* L.
SATYRIDAE	*Lethe verma* Koll., *Melanargia galathea* L.
AMATHUSIIDAE	*Taenaris phorcus* Westw.
BRASSOLIDAE	*Dynastor darius* Fab.
MORPHIDAE	*Morpho achilles* L.
NYMPHALIDAE	*Vanessa atalanta* L., *Neptis varmona* Mr.
RIODINIDAE	*Thisbe irenea* Stoll., *Diophthalma lagora* H.S.
LYCAENIDAE	*Megalopalpus zymna* D. & H., *Lycaenopsis akasa* Horsf.
PAPILIONIDAE	*Papilio polytes* L., *P. aegeus* Don., *Graphium antiphates* Cr., *Atrophaneura polydorus* L., *Parnassius delius* Esp.
HESPERIIDAE	*Milanion leucaspis* Mab., *Tagiades menaka* Mr.

possessing pigments related to uric acid.

The six PIERINAE and the two TERACOLINAE examined all have a white ground-colour. So have two of the DISMORPHIINAE (*L. sinapis* and *D. thermesia*), and the white variety of the female of *Colias croceus* (COLIADINAE) which was also tested. It seems clear that leucopterin is present in all of them. The three COLIADINAE (including the normal form of *C. croceus*) are yellow butterflies, and their reactions are similar to one another on exposure to chlorine. The yellow is immediately changed to white, and we may fairly assume that the xanthopterin is oxidised to leucopterin, for they then behave as do the white species already mentioned : the purple colour appears after considerable delay, but it does so rapidly on fuming with ammonia.

Hebomoia glaucippe has orange tips to the fore wings, while *Enantia melite* has an orange ground-colour. This orange shade must be due to a slight modification only of xanthopterin, for it behaves on exposure to chlorine exactly as does the true xanthopterin of *Gonepteryx rhamni*. *Pseudopieris nehemia*, *Enantia licinia*, and *Dismorphia nemesis* are pale yellow or cream coloured. They also become white on exposure to chlorine and subsequently develop a murexide effect, so that the pigments concerned are probably either mixtures of xanthopterin and leucopterin or else substances of similar structure.

The purple colour obtained in the DISMORPHIINAE is very much fainter than in the other subfamilies, which accords with the fact that this group is in many respects distinct from the rest of the PIERIDAE. I have shown that some of the DISMORPHIINAE (including *L. sinapis*, *P. nehemia*, *E. licinia*, and *E. melite*) possess flavone pigments (2). However, they also develop a murexide effect after treatment with chlorine, since their anthoxanthins do not replace, but are present in addition to, pigments of the uric acid series.

The species listed in Table 2 all possess some white pigment, but several have yellow markings in addition (*e.g.*, *Taenaris phorcus*). Not only is there no sign of a murexide effect to be detected in these insects, but such yellow pigments do not become white on exposure to chlorine.

The chlorine used in the majority of these tests was made from bleaching powder by treatment with hydrochloric acid, consequently it was moist. This did not affect the result, for I obtained identical reactions (with *Pieris napi*) when the chlorine, whether produced in this way or taken from a commercial cylinder, was dried by bubbling through concentrated sulphuric acid.

The delay in the reaction when chlorine is used alone was at first a puzzling feature of the murexide test here described. It is now clear that this is due to the time required for the specimen to reach a sufficiently alkaline state after treatment, therefore it occurs rapidly on fuming with ammonia. For when once produced, the purple colour disappears in a few minutes when the wings are acidified (*e.g.*, by exposure to hydrochloric acid vapour), but it can then be quickly recovered again by fuming with ammonia. Indeed, the purple derivative of leucopterin behaves, and could almost be used, as an indicator.

I discussed these results with the Baron de Worms, who kindly made

[1] Kindly supplied by Messrs. Organon Ltd. Laboratories.

corresponding tests with synthetic xanthopterin and leucopterin.[1] He found that chlorine oxidised synthetic xanthopterin to leucopterin, and he obtained a distinct though weak purple colour on subjecting synthetic leucopterin to chlorine and subsequently fuming with ammonia.

Fischer (1) reports that he successfully used chlorine in place of concentrated nitric acid in the normal murexide reaction. However, I detected no result with human urine corresponding to that observed with leucopterin. The urine was concentrated by heating (on a water bath) to a quarter of its original volume. It was then soaked up on strips of filter paper and allowed to dry. No murexide effect was produced when these strips were exposed to chlorine, or to chlorine followed by ammonia. It is possible therefore that this test distinguishes leucopterin from some of the other uric acid derivatives. Koschara (4) has isolated traces of xanthopterin from human urine. Presumably chlorine failed to distinguish this because of its low concentration.

In addition to chlorine, I have tested all the species included in Tables 1 and 2 with bromine, both alone and followed by fuming with ammonia. The result is entirely different from that due to treatment with chlorine ; for no purple colour of the murexide reaction appears, nor does bromine change xanthopterin into leucopterin. I have also examined the action of iodine vapour on the white pigment of *Pieris napi* and the yellow one of *Gonepteryx rhamni*, again with negative results.

The test here described is sufficiently sensitive to allow the detection of leucopterin even in a few scales when the wing of a butterfly is examined with a dissecting microscope.

I have in the course of this work received encouragement and helpful suggestions from Professor J. B. S. Haldane, Dr. Antoinette Pirie, of the Nuffield Laboratory of Ophthalmology, Oxford, and Baron Charles de Worms, for which I am most grateful. I am also indebted to Professor G. D. Hale Carpenter for his kind permission to use specimens from the Hope Department of Entomology, Oxford, for these chemical studies.

Summary.

1. A murexide test is described which can be applied to the pigments of intact insects.

2. The white and yellow colours of the Pieridae (Lepidoptera) are due to compounds related to uric acid. Their white pigment is leucopterin ($C_6H_5O_3N_5$). If a wing pigmented with this substance is exposed to chlorine for twenty minutes, a brilliant murexide purple develops after a delay of a day or two.

3. This delay is due to the time required for the treated specimen to reach a sufficiently alkaline state. The change can therefore be greatly hastened by fuming with ammonia after treatment with chlorine, when the purple begins to appear almost immediately. The murexide effect disappears on subsequent exposure to acid, but can once more be

recovered if the wing is again made alkaline, the purple derivative of leucopterin behaving as an indicator.

4. The yellow pigment of the PIERIDAE is xanthopterin ($C_6H_5O_2N^9$). This immediately becomes white on exposure to chlorine, being oxidised to leucopterin. It then develops the murexide reaction as described.

5. Hopkins had shown that while the white and yellow pigments of the PIERIDAE are apparently always uric acid derivatives, those of other butterflies are not.

6. The chlorine test has been applied to 17 species of the family PIERIDAE, distributed among each of its four main subfamilies. All of them gave a positive reaction.

7. Twenty species not belonging to the PIERIDAE were also tested. They were distributed among each of the ten other families of butterflies. None of them gave a positive reaction.

8. The chlorine test does not give a positive reaction with urine. It may therefore distinguish the pterins from certain of the other uric acid derivatives.

9. This murexide reaction is not obtained when bromine or iodine, whether alone or followed by ammonia, are used in place of chlorine. Neither bromine nor iodine oxidise xanthopterin to leucopterin.

10. The chlorine test for pterins is a sensitive one, allowing the recognition of these substances even in a few scales.

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STUDIES ON THE CHEMISTRY OF PIGMENTS IN THE LEPIDOPTERA, WITH REFERENCE TO THEIR BEARING ON SYSTEMATICS. 5. *PSEUDOPONTIA PARADOXA* FELDER

By E. B. Ford, F.R.S.

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The affinities of *Pseudopontia paradoxa* Felder are exceedingly obscure. Thus Aurivillius (1910) says that *Pseudopontia* Plotz, of which this is the only known species[2], is "the most peculiar of all known genera of butterflies. The differences in the build of the body are so great that some authors have placed the genus among the Heterocera." The structure of the imago has been studied in some detail, with the result that this insect has now been included in the Pieridae (Dixey, 1922; Tillyard, 1922); but it is so highly abnormal that this decision was reached only after considerable discussion. The antennae are clubless, though Jordan (1898) finds that they are of a pierine type in other respects. The neuration and the form of the palpi are very exceptional (Dixey, *ibid.*). So is the genital armature of the male, which has been examined by Eltringham (1922), who says that it is "of a peculiar structure and unlike that of any other species known to me. . . . The whole structure of these organs gives no clue to the systematic position of the species." However, he rightly adds, "In my opinion, the structure of the male armature is rarely to be relied on as an indication of more than specific affinity." *P. paradoxa* is limited in its distribution to the forests of the western equatorial region of Africa, where it is probably fairly common, though it is not a species with which collectors are generally familiar. Unfortunately, the early stages, which should throw much light on its affinities, are unknown in nature. Eltringham (*ibid.*) succeeded in dissecting out eggs from set specimens, but the results of his investigation of them are not very conclusive. He says that "though not typically pierine in form they at least resemble the eggs of that family more than those of others so far as they are known to me, and to that extent support the view that *P. paradoxa* is an aberrant Pierine species." Thus though apparently a member of the Pieridae, *Pseudopontia* is yet so distinct that it has been necessary to place it alone in a separate subfamily, the Pseudopontiinae E. Reuter, created to receive it; whereas the remaining subfamilies of the Pieridae, four in number, all contain scores, and the Pierinae hundreds, of species.

It is evidently a matter of considerable interest for the theory of classification to determine whether new and independent evidence of a distinct kind upholds the conclusions reached by the more usual

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1 The inclusion of a second supposed species, *cepheus* Ehrman., in this genus is a complete mistake (Dixey, 1922).

systematic methods when applied to so exceptional a butterfly as this. Such an independent test is provided by a study of the chemistry of its pigments.

Ever since the pioneer work of Hopkins (1895), it has been held that the white and yellow pigments of the PIERIDAE are pterins, built up from uric acid. I have indeed shown that this view requires slight modification, since anthoxanthins, which are of an exceedingly different nature, being non-nitrogenous, contribute to the production of these colours in a few members of this family (Ford, 1941). But when found, anthoxanthins are present in addition to, not in place of, the pterins (Ford, 1947). These uric acid derivatives therefore appear to be universal in the PIERIDAE. Moreover, so far as at present known, they are absent from other butterflies, even from the allied family PAPILIONIDAE, a fact indicated by Hopkins and recently confirmed on more extensive data (Ford, 1947). Consequently, we have every reason to believe that the presence or absence of pterin pigments is a criterion distinguishing the PIERIDAE from other families, so that it is of considerable importance to discover whether or not these substances occur in *Pseudopontia*.

Such an investigation has only recently become possible. The wings of *P. paradoxa* are nearly transparent and bear only a thin scattering of white scales. A large number of specimens would therefore have to be sacrificed for an ordinary murexide test and, as the butterfly is something of a rarity in collections, it would not be possible to obtain sufficient material for the purpose. However, the chlorine method for distinguishing pterins which has just been described (Ford, 1947) is so sensitive that it seemed worth while to apply it to this insect. By means of it, I was able to obtain a clear murexide reaction from a single wing of *Pseudopontia paradoxa*, so demonstrating the presence of pterin pigment in this species and consequently confirming, on entirely independent evidence, its inclusion in the PIERIDAE.

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OBSERVATIONS ON *SYSTOECHUS SOMALI* (DIPTERA BOMBYLIIDAE) ATTACKING THE EGGS OF THE DESERT LOCUST (*SCHISTOCERCA GREGARIA* (FORSKÅL)) IN SOMALIA

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During May and June, 1945, at the beginning of an anti-locust campaign in the Gabredarre district (Ogaden Province) of Somalia (*ex* Italian Somaliland), it was noticed that in many places the eggs of *Schistocerca gregaria* (Forskål) were being destroyed by a fly larva belonging to the family Bombyliidae (fig. 1, A & D). As it was at the time unknown if any species of Bombyliid had been recorded from the eggs of *S. gregaria* and as the only fly recorded as destroying locust eggs in Somalia was *Stomatorrhina lunata* Fabricius (Calliphoridae), it was decided to make such observations as were possible. Unfortunately, as I was constantly on the move under camp conditions, and the period between laying and hatching of locust eggs was a very busy one for Locust Control personnel, the information is incomplete. It has, however, been thought worth while to set down such as has been obtained.

Adult flies were submitted to Dr. B. P. Uvarov of the Anti-Locust Research Centre, to whom I am indebted for his interest and encouragement, and he arranged for their identification. It transpires that they belong to a new species of *Systoechus*, which Mr. H. Oldroyd (1947) has kindly described as *Systoechus somali* sp.n. Other species of *Systoechus* have been recorded as destroying the eggs of grasshoppers and other species of locusts in Russia, Siberia, North America and South Africa (Uvarov, 1928 ; Potgieter, 1929).

Field Observations.

The larvae were first found as typical third-stage Bombyliid larvae (Clausen, 1940) five or more days after the locust eggs had been laid. They were usually of varying size (2–14 mm. overall length), giving the impression that, in any one egg-packet, they were not all of the same age. No first or second stage larvae were seen. Ten or eleven days after the laying a large proportion of the larvae were already full-grown. The numbers of larvae in each egg-packet, each of which contained about 70 locust eggs, varied from 0 to over 30, but in most cases there were less then 10. Similar observations were made by Potgieter on the larvae of *S. albidus* Loew found in the egg packets of *Locustana pardalina* Walker. Most of the larvae of *S. somali* were found with their mouth parts applied to the middle of a locust egg, and that egg, and usually others nearby, was shrunken. It seemed that about 5 or 6 larvae were necessary to destroy all 70 eggs in the packet, but it is not known whether, when a larger number of larvae were present, all were able to

become full-grown. After 13–15 days any remaining locust eggs hatched, and the larvae remained *in situ*. At this stage they were all presumably sufficiently grown to mature, as no more food appeared to be available, but the variation in size was considerable (6–16 mm. overall length). After a few days the larvae, which had been translucent white, became more opaque and yellow, burrowed a short distance (1–2 cm.) from the old egg-packet, and remained in small ovoid cavities, similar to those described for *S. albidus* by Potgieter, 5–10 cm. below the soil surface. By this time (one week after the locusts had hatched), the soil was becoming dry and caked, and it continued dry for months. The normal interval between rains in Somalia is four to five months. Two months after the locusts had hatched the larvae were unchanged, except that they had become somewhat shrunken (fig. 1, *B*). They were still active when disturbed, but were obviously in diapause.

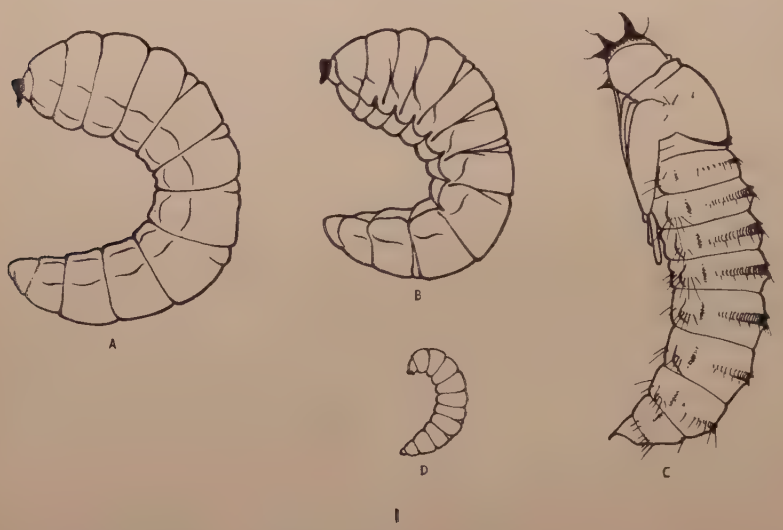

Fig. 1.—*Systoechus somali*. A full-grown larva. *B*, full-grown larva in diapause ; *C*, pupa ; *D*, young larva.

Although locust eggs were distributed over a wide area in Somalia, both during May–June, 1945, and October–December, 1945, on both layings *S. somali* larvae only occurred in large numbers in one relatively small area, which was much the same for both layings. This is shown, very roughly, in fig. 2, which is largely based on generalised and incomplete information from Locust Control personnel. Odd specimens and reports of a few larvae were obtained from many parts of Somalia, but nowhere, except in the area shown, were they common. The reason for this is unknown. Somalia is, generally speaking, remarkably uniform in altitude, climate, soil and vegetation, but it is noticeable that this is one of the few areas that is always laid over if locusts lay at all in the colony. Unfortunately, I was unable to visit it during the October to

December laying, but reports and specimens from Locust Control personnel confirmed my general observations, made in May to June. During those months, in some localities within the area, the locust eggs were completely destroyed, but in others destruction was only 5–20 per cent. of the egg-packets. This appeared to be correlated with the hardness of the soil, the heavier damage occurring in hard soils and the lighter in soft soils. In very soft sandy soils the larvae were almost absent. Near Wardere, at Afdub (6° 49′ N., 45° 7′ E.) the soil is patchy, consisting of hard red clayey soil with soft sandy patches. In late May the locusts had laid heavily and fairly evenly, and in early June, eleven days after the laying, I found that 96 per cent. of the egg-packets in the hard soil were infested, with an average number of 4·0 larvae per packet, while in the sandy soil only 32 per cent. were infested, with an average of 1·6 larvae per packet. Some places were even more heavily infested than the hard soil at Afdub, *e.g.*, near Gabredarre, at Fernard

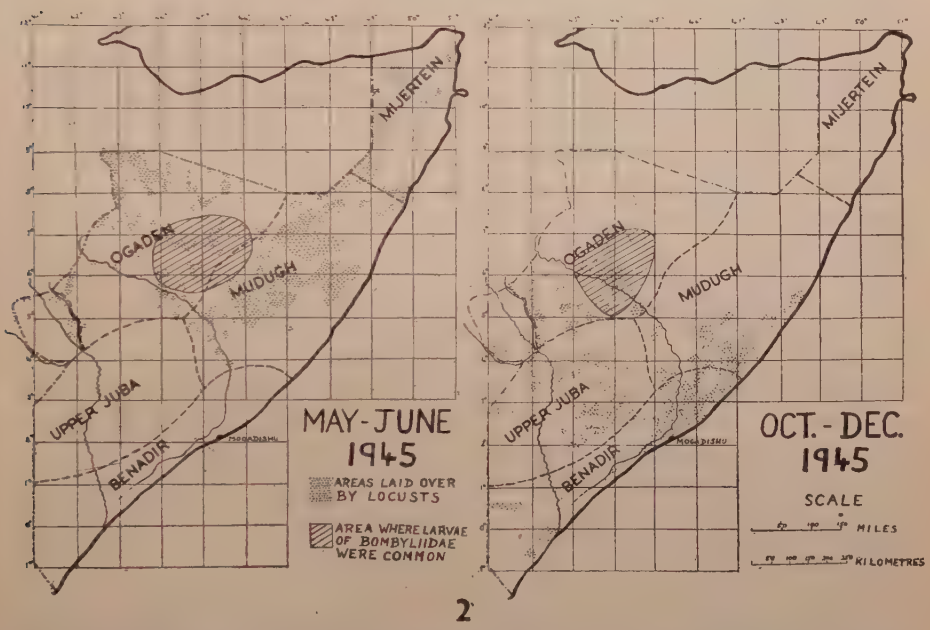

Fig. 2.—Maps of Somalia showing provinces, areas laid over by locusts during two seasons and, for both seasons, the general area where the larvae of *Systoechus somali* were common.

(6° 52′ N., 44° 18′ E.) 100 per cent. of the packets were infested, with an average of 18·0 larvae per packet; no locust hoppers hatched there. During the October to December laying, near Gabredarre 254 square miles of country were reported as laid over, in eight different areas, but relatively very few locust hoppers hatched. The Italian Locust Assistant, Signor Livio Pesenti, reported that in the soft soils approximately 40 per cent. of the eggs were destroyed, and in the hard soils 100 per cent. Elsewhere in the same general area (fig. 2) heavy damage was reported.

No data were obtained in the field about pupation or the adult flies, but some information about the former and specimens of the latter were obtained in rearing experiments. The fly was never seen in the field, but this is probably because I was not able to visit the heavily infested area at the right time. Other Bombyliids were frequently seen on flowers in various parts of Somalia.

Laboratory Observations.

A large number of larvae of *S. somali* in diapause were collected at Afdub on 31st July, 1945, from a locust egg-site laid over on 23rd May, and were taken back in dry soil to Mogadishu. On 13th August some of these larvae were divided into four lots, each of about 350 larvae, and were placed in four cages, each with a floor area of about 15 in. by 15 in. The floor of each cage was a square metal dish about 2 in. deep, and pierced at the bottom to allow free drainage. Each dish was filled with dry soil from Afdub and the larvae were buried. The cages were kept indoors where the temperature was fairly uniform (about 75°–85° F.) and no sunshine reached them. On August 13th cage A was heavily watered, so that the soil was saturated, and a few days later adults started to emerge. On September 12th cages B and C were watered, B being thoroughly saturated, while in C merely the surface of the soil was wetted. Thereafter, at approximately monthly intervals, B was watered heavily, and C lightly, while the soil in A was always kept saturated and that in D remained air-dry. The cages were examined daily for over a year, and records of the flies emerging were kept. Only 41 flies emerged, and at the end of the experiment many of the larvae were still alive and in diapause. I am indebted to Signor Giuseppe Saracini, who kept up the observations when I was absent from Mogadishu. The results are shown graphically in fig. 3, from which it can be seen that :—(a) No flies emerged in the dry cage, D, but that fifteen months after the laying of the locust eggs on which they had fed, and over a year after collection, over 40 per cent. of the larvae were alive. (b) Only three flies emerged from cage C, which was lightly watered at monthly intervals, and over 30 per cent. of the larvae were still alive at the end of the experiment. (c) Flies emerged in fair numbers from the two heavily watered cages, A and B, but mainly at two definite seasons (these correspond roughly with the two normal rainy seasons of the hinterland of Somalia), and many larvae survived until the end of the experiment without pupating. (d) The mortality in cage A, which was kept continually wet, was higher than in the other three cages, which were dry for at least some of the time.

It would appear therefore that water, of which, in the field, the only source is rain, is necessary to break the larval diapause, but also that emergence of adults only occurs during the normal rainy seasons, when locust egg-laying may reasonably be expected, even if rain should occur at other times (cages A and B). Also cage C indicates that light rain, which would not dampen the soil sufficiently to induce locusts to lay, does not cause the flies to emerge. The three flies that did emerge in cage C were possibly from larvae that were lying near the surface,

where they were dampened, and not at a depth of 5 or more cm. at which they would normally have occurred in the field. In Somalia locusts normally begin to lay in October to November, and again in May to June, and it is noticeable that, even under the uniform conditions of the experiment it was approximately at these times that almost all the flies emerged. It appears therefore that, apart from the necessity for heavy rain to cause fly emergence (and incidentally locust egg-laying), there is an underlying cycle, confining the ability to emerge roughly to the normal rainy season, and that if the rains fail altogether the larval diapause continues. It could be suggested that during the experiment the larvae were influenced by some other manifestation of the rainy season, but this is thought to be unlikely, as, apart from the fact that the cages were indoors, the climate and seasons of places, like Mogadishu, on the Somalia coast differ greatly from those even a few miles inland.

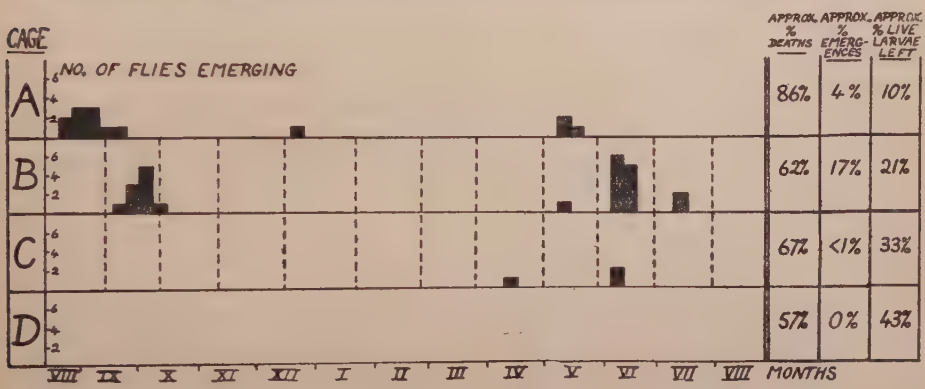

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FIG. 3.—Histogram showing number of adults emerging in four cages, week by week, from 13th August, 1945, to 28th August, 1946, and the percentage of deaths and percentage of larvae remaining in diapause after 28th August, 1946. The vertical broken lines represent the dates on which cages B and C were watered. For further explanation see text.

Another point of considerable interest is that even when conditions and time are favourable for emergence for some larvae, others continue in diapause. This phenomenon was also observed by Potgieter in *S. albidus*, and by de Lepiney and Mimeur (1930) in *Glossista infuscata* Meigen found in the egg-packets of *Dociostaurus maroccanus* Thunberg. Over 20 per cent. of the larvae in cage B remained as live larvae after two possible emergence periods and all these appeared healthy. Even in cage A, where the higher mortality indicated more unfavourable conditions, 10 per cent. remained. Larvae in diapause in dry soil were shrunken, but when the soil was wetted they all swelled to their original size, and those that did not pupate remained apparently unchanged for long periods, apart from a slight reduction in the area of the fat-body as observed through the skin. They were able to move actively at all times and to form new cavities in the soil.

It is unknown if *S. somali* will breed on the eggs of grasshoppers other than *S. gregaria*, but it is highly probable that it will do so, because between locust outbreaks *S. gregaria* is apparently quite absent from Somalia for periods of several years. On the other hand, the ability to remain in diapause through long periods even when conditions are favourable for emergence does suggest the unlikely possibility that *S. somali* may remain in diapause from outbreak to outbreak. In any event this ability probably does ensure that, when, as happened in May to June, 1946, no locusts lay in Somalia during a whole rainy season, many flies are held over until the next or later possible laying seasons. It would be interesting to observe if, in the absence of a locust laying during an outbreak, the local grasshoppers are seriously affected by *S. somali*, which could be presumed to have built up a large population on previous locust layings.

The pupae (fig. 1, *C*) were found to vary from 9 to 13 mm. overall length. They were formed in the larval soil cavities, and when ready to emerge they wriggled their way to the surface and pushed out the head and thorax. The flies then emerged rapidly, leaving the pupal skins with their abdomens still in the soil. Three flies were seen to emerge in the laboratory, at approximately 0900 hrs., 1300 hrs. and 1500 hrs. respectively, and indirect evidence indicated that in the laboratory emergences always occurred in the day time.

The pupal period appeared to be short, but it was not possible to determine its length by direct observation, as the larvae would not pupate in small tubes filled with soil. On seven occasions, however, larvae in diapause were watered and produced adults on the 9th, 10th, 10th, 10th, 11th, 13th, and 15th days respectively. It is certain therefore that it was less than 9–15 days under laboratory conditions. Once a cage which was producing adults was examined for pupae, and, although none was found, a fly emerged only two days later. It is possible, however, that a pupa was overlooked. Potgieter records the pupal period of *S. albidus* as 7–9 days in an incubator and 14–23 days outdoors in the summer ; de Lepiney and Mimeur record the pupal period of *G. infuscata* as 10 days.

It proved impossible to keep the adult flies alive in captivity as, unless they were kept undisturbed in the dark, they immediately battered themselves against the wire gauze of the cages or the glass sides of tubes.

The behaviour of ovipositing flies was observed in Turkana by Dr. D. L. Gunn, who has kindly permitted me to quote his unpublished observations as follows : "The behaviour of the Bombyliid was watched while it was apparently laying eggs into locust egg holes. The fly hovers over the hole in the sand left by a locust at a height of 1–3 cm. Something like an egg can be seen emerging from the extended abdomen, which is curved forward. The fly jerks forward and the presumed egg disappears. At the same time, a few grains of sand are disturbed at the entrance of the hole, as if by a projected egg. No eggs were recovered and no suitable larvae found four days later. The number of jerks per hole was between 10 and 20 in five cases and 40 in a sixth."

It is interesting to note that although many hundreds of full-grown larvae were kept in captivity for a long period no parasites were observed.

Acknowledgements.

Apart from acknowledgements already made above, I would like to express my thanks to all those members of the staff of Locust Control, Somalia, both European and native, who supplied me with information about the distribution of the larvae. Often the information was very vague and incomplete, but it did, I hope, give a general picture of the local distribution. I am particularly grateful to some of the native scouts who walked often for miles to bring in sticky handfuls of locust eggs with which a few limp larvae were entangled.

Specimens of larvae, pupae and adults have been deposited at the British Museum (Natural History).

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THE USE OF COMMON SALT FOR KEEPING DOWN VEGETABLE AND OTHER MOULDS DURING BREEDING EXPERIMENTS

By R. L. E. Ford, F.R.E.S.

In an earlier paper I mentioned that I had made the discovery that common salt (NaCl) would serve to keep down mould forming on vegetation in closed vessels when breeding of larvae was in progress. At the time I had not sufficient data available to say more, but have since conducted many comprehensive experiments, using altogether thousands of larvae, and can now show how this discovery may be put to practical use.

One of the chief nuisances met with when breeding large numbers of lepidopterous or other larvae, especially micro-lepidoptera, is mould forming on the leaves and on the larval excrement. This often causes a high rate of mortality and is a great handicap, since the obvious preventives such as proper spacing and air conditioning are often impracticable.

Especially is this the case when breeding micro-lepidoptera from flower blossoms, or rolled-up leaves, when doing parasite work. It is my practice to keep several hundred blossoms or leaves in a relatively small container, such as the earthenware pan described by me in 1943.

With the use of common salt in solution I can now obtain emergences up to 80 per cent. ; the remaining 20 per cent. being more probably the result of random collecting rather than mortality, since when collecting larvae in rolled leaves or blossoms it is impossible to examine each one individually.

My choice of NaCl is to make matters simple for all. Common household salt is available without any delay, not only in this country but nearly all the world over. However, there may be other salts which would serve as well or even better, but I leave it to others to explore this field. This brings me to suggest that something on these lines might serve to keep down fungus or even virus diseases common in Lepidoptera and other insects.

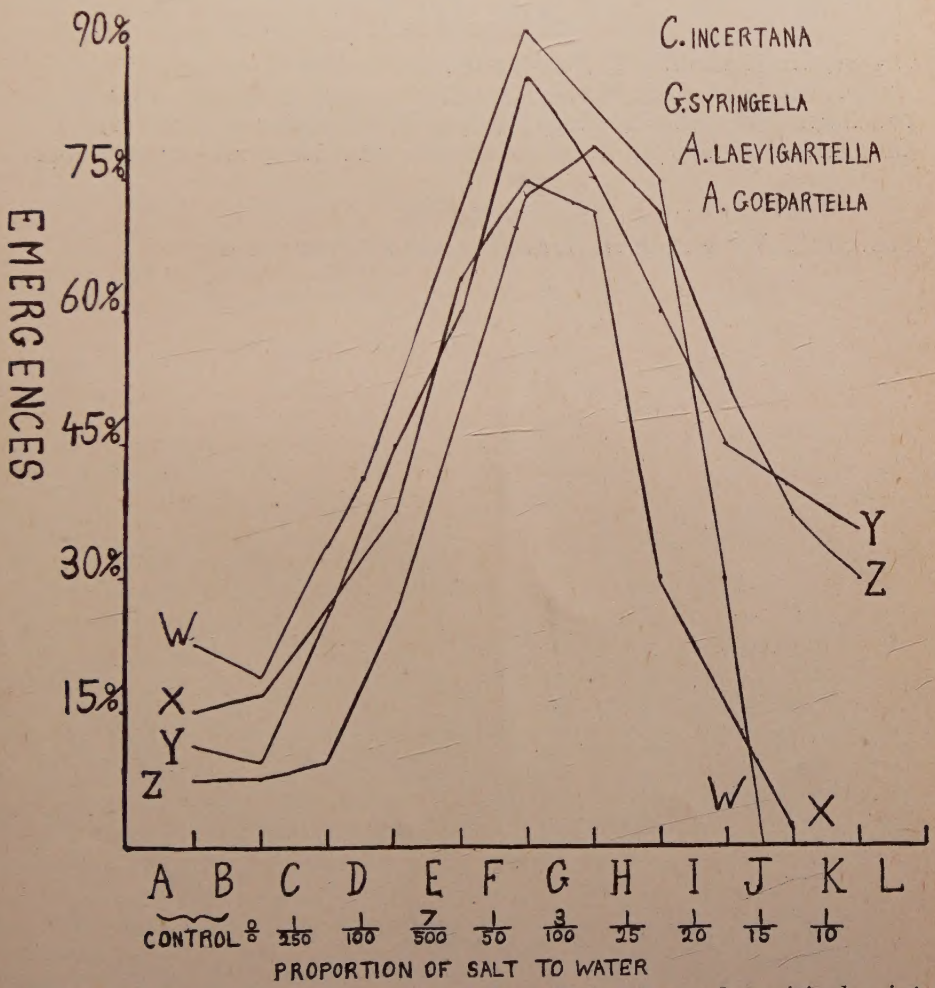
For the purpose of securing data I used twenty earthenware pans about 12 inches in diameter and 5 inches deep, having an inch of sterilised sand on the floor and covered by sheets of plate glass. Unglazed earthenware is essential.

I confined up to five hundred larvae all of the same species in each pan, using an equal number in each. I have converted the results into percentages for the sake of uniformity, but unfortunately this does not reveal the total of the enormous numbers used. Where figures for total emergences are given these include both Lepidoptera and their parasites. Since the parasites obtained were all solitary species this is quite fair, and I was able at the same time to work on the biology of a new *Microgaster* and some *Apanteles*.

The first two pans were left untouched except for the normal removal of superfluous moisture formed on the glass, by turning over the glass daily. The remaining pans were sprayed with varying solutions of common salt. The same strengths were repeated with each experiment. I have given the strengths of the various solutions in easy fractions for the sake of simplicity.

In some cases where the insects were emerging over a fairly long period it was necessary to give a second application.

The results obtained are shown below. It will be seen from these that an immediate improvement is effected even with a weak solution,



but after increasing the strength of the solution beyond a critical point the salt kills either the larvae or the pupae.

Finely granulated salt crystals sprinkled on the leaves do not deal with the mould so effectively and larvae coming into contact with them are killed. The examples chosen for the experiments are all species

feeding inside the leaves or spun up in blossoms, etc., and are therefore protected from direct spraying, and it is not suggested that foliage sprayed by the solutions could be safely eaten by all larvae. At the same time this method has been found most helpful and effective and has greatly advanced my breeding technique.

This method will also serve to keep down mould forming on larval excrement on the floor of breeding cages, when it is not possible to clean for some reason such as the risk of disturbance whilst moulting or pupation is taking place. When there is soil or pupating medium beneath, the larvae burrowing down are not affected.

Species Used.

Argyresthia goedartella L. (feeding in birch catkins).
Argyresthia laevigatella Herrich-Schäffer (feeding in shoots of larch).
Gracilaria syringella Fabricius (feeding in rolled leaves of privet).
Cnephasia incertana Treitsche (feeding in spun blossoms of buttercups).

Reference.

Ford, R. L. E., 1943, *Proc. R. ent. Soc. Lond.* (*A*) **18** : 89–94.

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1947

WEDNESDAY, October 1, 15

,, November 5, 19

,, December 3

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